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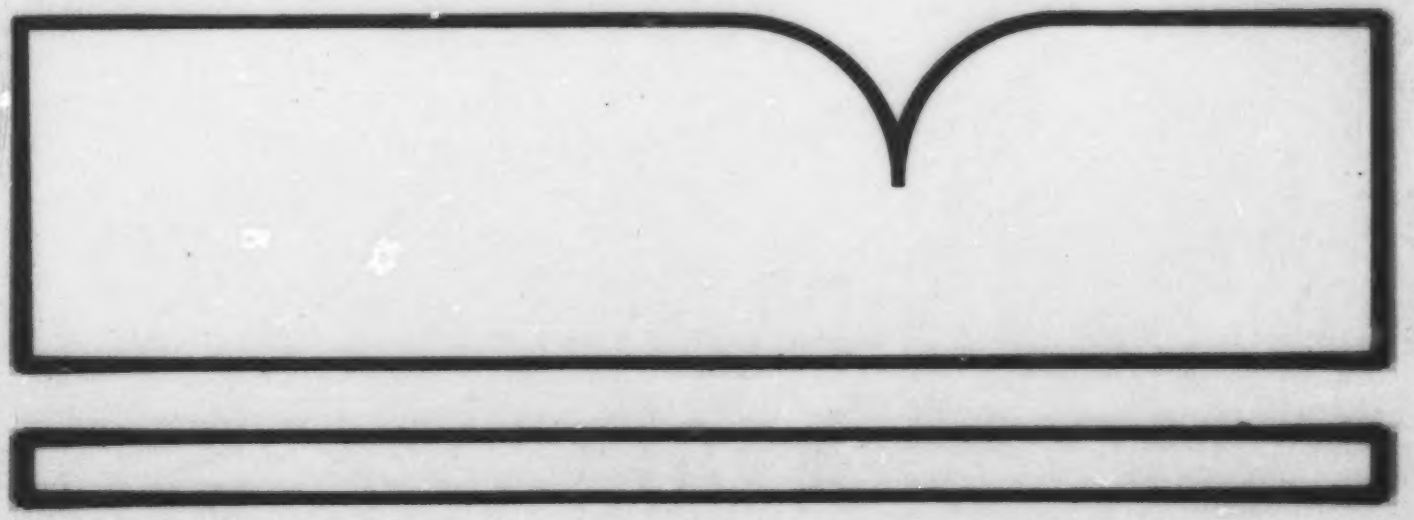

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A KARYOLOGICAL STUDY OF THE CALANOID COPEPOD *EURYTEMORA AFFINIS*

Pauline Vaas and Gerald G. Pesch

ABSTRACT

Chromosomes of the calanoid copepod *Eurytemora affinis* are described. The diploid chromosome number determined from cells at metaphase is twenty. There are ten pairs of metacentric chromosomes which can be divided into three size classes. No evidence of a heteromorphic chromosome pair was found. The method of tissue and slide preparation is described.

The genus *Eurytemora* contains approximately 15 species (Gurney, 1931). The chromosome complement of none of these species has been determined prior to this work, although genetic studies in the form of breeding experiments have been performed with *E. affinis* (Ketzner and Bradley, 1982). In general, few chromosome studies have been done on copepods. Of the entire order Calanoida, genetic studies have been performed on approximately 35 species. An excellent summary of genetic studies on both marine and freshwater copepods can be found in Colombera and Lazzaretto-Colombera (1978). More recent work, and work not included in the aforementioned study, has been published by Terpilowska (1974), Tsytsugina (1975), Frenzel (1978), and Chinnappa and Victor (1979).

In this paper, the results of a chromosome study of *E. affinis* are given. Also included is a description of the technique used for chromosome preparation. This technique, although used on other classes of animals (Kligerman and Bloom, 1977; Pesch and Pesch, 1980), is new in the study of copepods. Most other researchers concerned with copepod karyology have used the squash technique.

MATERIAL AND METHODS

Cultures of *E. affinis* were raised in the laboratory for several generations at 15°C in natural sea water diluted to 15‰ salinity with deionized water. A mixed algal diet was used, consisting of *Rhodomonas baltica, Skeletonema costatum, Tetraselmis suecia* (=*Platymonas suecia*), and *Pavlova lutheri* (=*Monochrysis lutheri*). Copepod stocks were obtained from Pettaquamscutt River, Narragansett, Rhode Island, with the use of a 153-μm plankton net.

The procedure for slide preparation is adapted from Kligerman and Bloom (1977). A mixture of all life stages of *E. affinis* was exposed to a solution of 5 mg colchicine in 100 ml of 15‰ sea water for 2 h at 15°C. The animals were pipetted from the colchicine solution and placed in 50 ml of 0.075 M potassium chloride for 1 h at room temperature. This was followed by fixation in 3 changes of ethanol-acetic acid (3:1) for 15 min each. Centrifugation (1,000 rpm for approximately 2 min) was necessary to condense the material before draining off the fixative between changes. The material was transferred to a small glass receptacle, covered with 1 ml of 50% glacial acetic acid, and macerated for 1 min. This produced a suspension of single cells which was then transferred to slides and allowed to air dry. The slides were stained for 10 min with 3% Giemsa in a buffered solution prepared with 0.1 M citric acid and 0.2 M disodium phosphate in deionized water adjusted to pH 7. After rinsing with

Table 1. Distribution of mitotic chromosome counts for *Eurytemora affinis*.

	Number of cells with the diploid chromosome number of:												
	12	13	14	15	16	17	18	19	20	21	22	23	24
	1	2	0	5	8	6	14	25	84	5	3	1	1
% of total	0.6	1.3	0	3.2	5.2	3.8	9.0	16.0	54.0	3.2	1.9	0.6	0.6

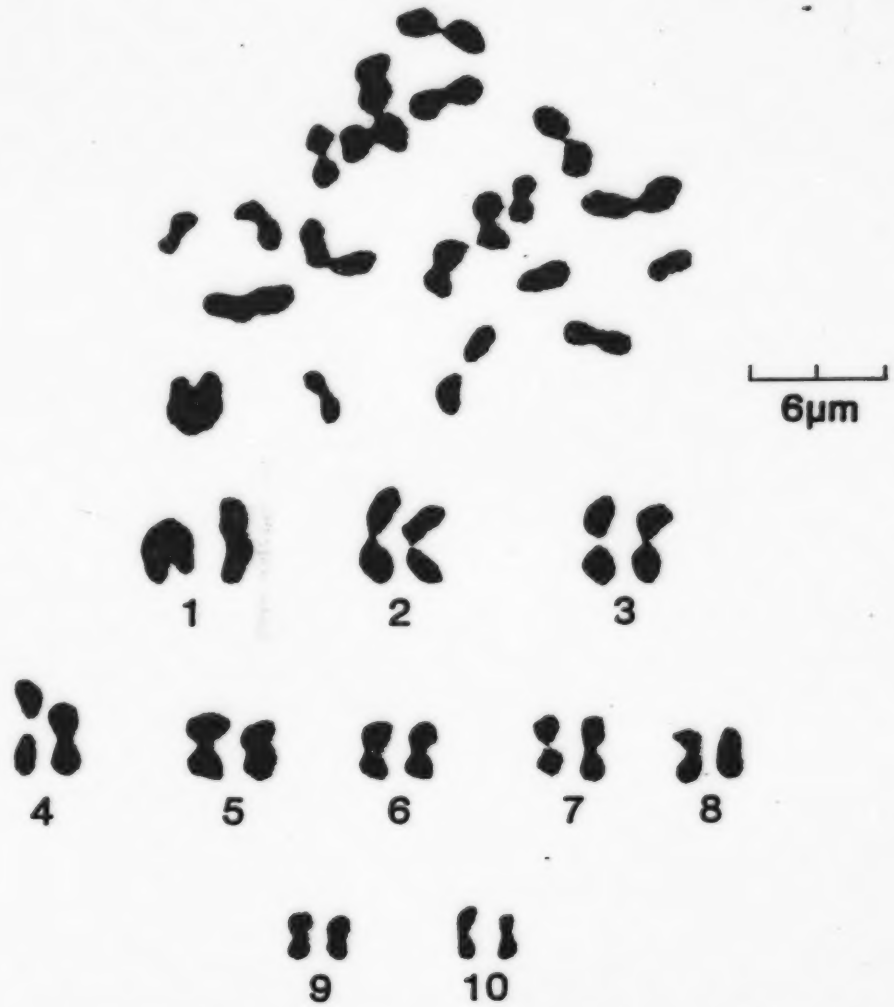

Fig. 1. Chromosomes of *Eurytemora affinis* paired and numbered from largest to smallest. The original set as seen under the microscope is shown above the numbered set.

deionized water to remove the excess stain, the slides were air dried. dipped in xylene, and mounted with Coverbond. (Mention of brand names does not imply endorsement by the United States Environmental Protection Agency.)

Photographs were taken with high contrast copy film at 1.000× magnification.

Results and Discussion

Chromosomes were counted in 155 cells arrested at metaphase of mitosis. Of these, 84 (54%) contained 20 chromosomes. The next most frequent number, 19.

was observed in 25 cells (16%). A chromosome number of 20, therefore, appears to be the diploid number for *E. affinis*. A distribution of chromosome counts is given in Table I. Variations in chromosome number are probably due to artifacts in slide preparation. Fragmented cells could have resulted in low counts and parts of two adjacent cells counted together could have caused high counts.

The chromosomes of *E. affinis* are shown in Fig. 1. All of the chromosomes are metacentric. This chromosome form is prevalent in the class Copepoda (Colombera and Lazzaretto-Colombera, 1972; Goswami and Goswami, 1974; Terpilowska, 1974; Chinnappa and Victor, 1979). The chromosomes can be classified by size into three groups: the first has three large pairs; the second has five medium pairs; and the third has two small pairs. The pairing of the chromosomes shown in Fig. 1 is based on visual judgement of size and is done to facilitate illustration of the three major size classes and the number of pairs.

It is impossible to determine the presence of an XY chromosome pair from the existing data; except for the cell shown in Fig. 1, none of the cells used for chromosome counts was resolved enough to see the exact shape of each chromosome. This does not preclude the presence of such a heteromorphic chromosome pair in *E. affinis*. Other authors have reported evidence of such chromosomes in certain species of copepods (Beerman, 1959; Rusch, 1960; Orlando, 1973; Goswami and Goswami, 1974). An XO mode of sex determination probably does not occur. If it did, the sex (male or female) which was determined by the presence of only one X chromosome (XO) would have 19 chromosomes, given that 20 would be the number for that sex (male or female) determined by the presence of two X chromosomes (XX). In this case, the ratio of cells containing 19 chromosomes to cells containing 20 chromosomes would be more nearly equal to the ratio of sexes in the laboratory-reared population of copepods, which is not the case (Martha Marcy, personal communication).

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